Metaecosystem dynamics drive community composition in experimental, multi-layered spatial networks

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Abstract

Cross-ecosystem subsidies are studied with a focus on resource exchange at local ecosystem boundaries. This perspective ignores regional dynamics that can emerge via constraints imposed by the landscape, potentially leading to spatially-dependent effects of subsidies and spatial feedbacks. Using miniaturized landscape analogues of river dendritic and terrestrial lattice spatial networks, we manipulated and studied resource exchange between the two whole networks. We found community composition in dendritic networks depended on the resource pulse from the lattice network, with the strength of this effect declining in larger downstream patches. In turn, this spatially-dependent effect imposed constraints on the lattice network with populations in that network reaching higher densities when connected to more central patches in the dendritic network. Consequently, localized cross-ecosystem fluxes, and their respective effects on recipient ecosystems, must be studied in a perspective taking into account the explicit spatial configuration of the landscape.

Keywords: meta-ecosystem, metacommunity, cross-ecosystem subsidy, allochthonous resource pulse, spatial networks, riverine dendritic networks

Introduction

The significance of cross-ecosystem subsidies in supporting recipient ecosystems is well recognized (Polis et al. 1997, Soininen et al. 2015, Richardson and Sato 2015). For instance, Fisher & Likens (1973) estimated that small streams obtain 75% of their total energy budget from terrestrial sources. Those cross-ecosystem subsidies can support complex communities (Polis and Hurd 1995), and sometimes lead to indirect bottom-up effects across ecosystems via spatial trophic dynamics (Knight et al. 2005, Bultman et al. 2014, Koel et al. 2019). Cross-ecosystem subsidy studies generally focus on population or community response to resource exchange at the ecotone between two ecosystems (Richardson and Sato 2015). This focus on local ecotone ignores regional scale dynamics that can emerge via dispersal and spatial feedbacks. More specifically, spatial feedbacks emerge because of the bi-directionality of cross-ecosystem resource exchange at the ecotone (Leroux and Loreau 2012), while dispersal can eventually extend the influence of local changes at much larger scale (Polis et al. 1997). The physical structure of the landscape is likely to constraint those processes by influencing both the effect of resource flows at the ecotone and organism movement at the landscape scale. The effect of landscape configuration on population-level (Altermatt and Fronhofer 2018) and community-level (Tscharntke et al. 2012, Tonkin et al. 2018a) processes are well-documented. However, cross-ecosystem dynamics scaling from localized resource flows at ecosystem boundaries to landscape-wide effects remain mostly unstudied (but see Schindler and Smits 2017; Lafage et al. 2019).

In a cross-ecosystem context, the landscape can be represented as two or more spatial networks embedded within one another and interacting through the exchange of cross-

ecosystem resources (Mucha et al. 2010) (Figure 1). In natural landscapes, dendritic river networks are connected with terrestrial spatial networks by the exchange of resources (organic matter, inorganic nutrients, Figure 1). Each network also undergoes its own internal spatial dynamics characterized by the movement of organisms through dispersal or foraging behaviors that are constrained by the shape of the network itself. This internal dynamic leads to intrinsic spatial variations in biodiversity within each network (Harvey and MacDougall 2014, Tonkin et al. 2018b). For instance, river networks have a highly conserved structure that is known to constrain alpha and beta diversity in contrasting ways compared to simpler lattice networks or unstructured (random) networks, generally leading to higher alpha and lower beta diversity in downstream than in upstream patches (Finn et al. 2011, Carrara et al. 2012, Tonkin et al. 2018b). Models and experiments have shown that those constraints on biodiversity can emerge from the topology of the dendritic network itself, which intrinsically drives the distribution of habitat capacity (i.e., volume or size) and dispersal limitation (Carrara et al. 2012). Assuming equal transfer of cross-ecosystem resource from the terrestrial network to the river network across the landscape, smaller and more isolated upstream patches should respond more to resource pulses than larger and more connected downstream patches, where the local effect of resource exchange is more likely to be diluted by larger volume. At the landscape scale, this would lead to spatial variation in the effects of cross-ecosystem resource exchange depending on the position in the river network.

Bi-directional resource exchange between two spatial networks could also lead to spatially-dependent feedbacks where, for instance, effects from the terrestrial to the river network are disproportionally important in upstream reaches (large increase or decrease

66 in detritus production, for reasons described above), in turn, leading to effects on the
67 terrestrial network being especially important for terrestrial patches connected to
68 upstream sites in the river network (Figure 1). Thus, via bi-directional cross-ecosystem
69 resource exchange, one of the spatial networks can potentially impose their own
70 structural constraints on the connected network leading to a "mirroring effect"
71 influencing the entire spatial network but only visible at landscape extent.

72 In this study, our main objectives were to test **i)** the influence of the position in the
73 landscape on the effects of cross-ecosystem resource exchange and, subsequently, **ii**) if
74 and how spatial feedbacks emerge at the landscape scale, leading to a "mirroring effect"
75 on population dynamics and community composition. Based on the information
76 mentioned above, our two main working hypotheses were that H1) Habitat size will
77 modulate the effect of subsidies and H2) Constraints related to spatial configuration in
78 one network will be reflected in the connected network via spatial feedbacks (Figure 1).
79 More specifically our experiment was aimed at testing the two specific predictions that
80 P1) The effect of resource exchange will be stronger in smaller and less connected
81 relative to larger and more connected patches and P2) The effect in P1 will be also
82 detected in the other connected network with patches connected to smaller and less
83 connected patches supporting lower species densities than patches connected to larger
84 and more connected patches.

85 To test those predictions, we used microcosms in a controlled laboratory experiment,
86 building miniaturized spatial network analogues, reflecting the general spatial properties
87 of natural landscapes. Specifically, we connected a dendritic spatial network
88 (representative of riverine networks – "blue network") to a 4-nearest neighbor lattice

network (representative of a terrestrial matrix embedding the river network – "green network") by the exchange of resources (Figure 1). Each network was composed of local ecosystems (i.e., each microcosm) connected by the dispersal of living organisms along the specific structure of the respective network (Figure 1). At the local scale, each of these local ecosystems was also linked to a local ecosystem in the other network (i.e., across the blue and green network) by the exchange of resource only (i.e., inorganic nutrients and dead biomass). Thus, living organisms were moving only within each network, while dead biomass also moved between the two networks. The blue network contained seven interacting bactivorous protist species while the green network contained bacterial communities. This disparity in biotic complexity between the two networks made the bi-directional effect of resource exchange more tractable to test our working hypotheses. We focus on the influence of resource pulses from the green network on protist community dynamic in the blue network and, in turn, on how the altered dynamics along the blue network might feedback and affect the spatial distribution of bacterial densities in the green network compared to isolated controls ("mirroring effect", Figure 1). Our large-scale (504 microcosms) experiment replicated across entire landscape-analogues allowed us to test how the landscape *per se* constrains spatial variation in the effect of cross-ecosystem resource exchange and how it scales up to generate regional-level patterns.

111 **Methods**

112 Each network ('blue' and 'green') was represented by 36 microcosms connected by
113 dispersal along its edges (Figure 1). We had four replicates of each network connected by
114 resource exchange (288 microcosms). Then, as controls, we had four isolated (not
115 connected to a green network) replicates of the blue network (144 microcosms) and two
116 isolated (not connected to a blue network) replicates of the green network (72
117 microcosms –spatially homogenous dispersal). In total, we had 504 microcosms. The
118 experiment lasted 29 days with 5 sampling events.

119 For the green network, we used a square lattice network (Figure 1). This choice was
120 justified by the many previous theoretical and empirical metacommunity studies using
121 simplified lattice networks to approximate connectivity and dispersal based on Euclidean
122 distances in many terrestrial '2D' systems (Hanski and Gaggiotti 2004, Leibold et al.
123 2004, Holyoak et al. 2005).

124 For the blue network, our four replicates corresponded to four different realizations of
125 dendritic networks generated from four different space-filling optimal channel networks
126 (Rigon et al. 1993). Optimal channel networks are known to reproduce the scaling
127 properties observed in river systems (Rinaldo et al. 2006, Carrara et al. 2014). They are
128 built under the assumption that drainage network configurations should minimize total
129 energy dissipation, and the empirical observation that river network properties constitute
130 scale-invariant fractals (Rinaldo et al. 2006). To reduce the four networks generated this
131 way (corresponding to our four replicates for the blue network) to a logistically possible
132 level for a laboratory experiment, a coarse-graining procedure was used to generate 6x6

133 patch networks of four different volumes (7.5, 13, 22.5 and 45 mL), preserving the
134 characteristics of the original three-dimensional basins (Rodriguez-Iturbe and Rinaldo
135 1997, Carrara et al. 2014).

136 Biotic communities in the blue network were composed of six bacterivorous protist
137 and one rotifer species (henceforth called "protists"): *Tetrahymena* sp. (Tet), *Paramecium*
138 *caudatum* (Pca), *Colpidium striatum* (Col), *Spirostomum* sp. (Spi), and *Chilomonas* sp.
139 (Chi), *Blepharisma* sp. (Ble) and the rotifer *Cephalodella* sp. (Rot). The latter two species
140 can also to a lesser degree predate on smaller protists. The protists were feeding on a
141 common pool of bacteria (*Serratia fonticola*, *Bacillus subtilis* and *Brevibacillus brevis*).
142 Prior to the beginning of the experiment, each protist species was grown in monoculture
143 in a solution of pre-autoclaved standard protist pellet medium (Carolina Biological
144 Supply, Burlington NC, USA, 0.46 g protist pellets 1 L^{-1} tap water) and 10% bacteria
145 inoculum, until they reached carrying capacity (for methodological details and protocols
146 see Altermatt *et al.* 2015).

147 Each ecosystem in the green network was set at 10 mL. Biotic communities in the
148 green network were composed of three bacteria species (*Serratia fonticola*, *Bacillus*
149 *subtilis* and *Brevibacillus brevis*). It is noteworthy that we initially inoculated the green
150 network also with an autotrophic protist species (*Euglena gracilis*). However, the species
151 did not establish well in the network and all individuals died before or very soon after the
152 start of the experiment. Because the species was inoculated at equal density in each
153 ecosystem of the green network, we can safely assume that the death of all individuals
154 did not generate significant within network variations in detritus (and considering the

155 additional homogenizing effect of dispersal). For this reason, we assumed a zero-sum
156 effect, and will not consider this species further.

157 Each ecosystem consisted of a 50 (blue networks) or 15 (green networks) mL
158 polypropylene Falcon tube (VWR, Dietikon, Switzerland). At day 0, we pipetted an equal
159 mixture of each of the seven protist species at carrying capacity into each ecosystem of
160 the blue network to reach the corresponding volume (7.5 mL, 13 mL, 22.5 mL, 45 mL).
161 Communities were allowed to grow 24 hours before the first dispersal event. Within-
162 network dispersal and cross-ecosystem resource exchange occurred twice a week, while
163 sampling of the communities for species count was done once a week (two
164 dispersal/resource pulse events between each sampling with at least 48 hours between the
165 last dispersal/resource pulse event and sampling). Sampling events and counting were
166 done at day 0, 7, 15, 21, 29 of the experiment, while dispersal and cross-ecosystem
167 resource pulse events occurred at day 1, 4, 8, 11, 16, 19, 22, 25 of the experiment. On the
168 dispersal/resource pulse days, dispersal was always done first, so that the pulsed resource
169 added to each patch would stay in that patch until the next dispersal event.

170 Dispersal was done by pipetting a fixed volume from one ecosystem to each of the
171 connected ecosystems along the edges of the spatial network, using mirror networks
172 (following methods developed in Carrara *et al.* 2012). We assumed higher dispersal in the
173 blue (1 mL) compared to the green (0.5 mL) network to mimic the action of physical
174 flows in riverine dendritic networks. Dispersal was bi-directional along each edge for
175 both networks (e.g., 1 or 0.5 mL from ecosystem a to b and 1 or 0.5 mL from ecosystem
176 b to a), which ensured the maintenance of the same volume in each ecosystem throughout
177 the 29 days of the experiment. We implemented bi-directional dispersal to avoid the

178 logistical challenge of maintaining equal ecosystem volumes. Thus, we were testing only
179 for the structural property of the riverine spatial network. Previous work has shown that
180 in many cases those structural constraints related to hierarchical patch size distribution
181 are sufficient to replicate biodiversity patterns found in nature (see Carrara et al., 2014).
182 In a previous study, we also conducted a sensitivity analysis including an extensive
183 number of simulations to confirm that this experimental assumption on dispersal did not
184 affect the network effects on species richness (see Appendix A and Figs. S2 & S3 in
185 Harvey et al. 2018).

186 Cross-ecosystem resource pulse was done by exchanging dead biomass from one
187 network to the other. First, a set volume was removed from each ecosystem in the blue (1
188 mL) and green (1.25 mL, see paragraph below for explanation on the volume difference)
189 networks. Those volumes were then microwaved until boiling to turn all living cells into
190 detritus (following methods developed in Harvey et al. 2016, 2017). After a cooling
191 period, the microwaved samples were poured into the specific recipient ecosystem in the
192 recipient network (see Figure 1). To control for the mortality effect, we performed the
193 same steps of sampling and microwaving in the isolated control networks, with the
194 difference that the microwaved volume was poured back to the ecosystem of origin.

195 At each measurement day, sampling was done by pipetting a total of 0.5 mL from each
196 ecosystem of each network that was then used to measure bacteria (0.1 mL) and protist
197 densities (0.4 mL). Removing 0.5 mL from microcosms in the blue network will have
198 different impacts depending on ecosystem volume. For this reason, we compensated this
199 volume lost on a weekly basis by exchanging 0.25 mL more volume from the green to the
200 blue network (resource exchange is done 2 times/week, thus totally replacing the 0.5

mL). Protist abundance was measured by using a standardized video recording and analysis procedure (Pennekamp and Schtickzelle 2013, Pennekamp et al. 2015). In brief, a constant volume (34.4 μL) of each 0.4 mL sample was measured under a dissecting microscope connected to a camera for the recording of videos (5 s per video). Then, using the R-package bemovi (Pennekamp et al. 2015), we used an image processing software (ImageJ, National Institute of Health, USA) to extract the number of moving organisms per video frame along with a suite of different traits for each occurrence (e.g., speed, shape, size) that could then be used to filter out background movement noise (e.g., particles from the medium) and to identify species in a mixture (details were published in Appendix C of Harvey *et al.* 2018). Finally, for bacteria we measured densities using standard flow cytometry on fresh SYBR green fixated cells using a BD Accuri^{TM} C6 cell counter (1/1000 dilution). For logistical reasons and because of time constraints, bacteria counts were only done for two of the four replicates in blue and green networks.

Statistical analysis

The main objective of this experiment was to identify landscape-scale feedbacks between the two spatial networks connected by the pulse exchange of resources. Our focus was on the interaction term between position in the blue network and the resource pulse treatment. Our second main working hypothesis was the “mirroring” effect where we expected to find an imprint of the blue network within the green network (Figure 1).

Effects from green to blue network

222 Our main response variable in the blue network was changes in protist community
223 composition (i.e., abundance and occurrence) because it encompasses effects on both
224 diversity and the more subtle influences on the structure and functioning of the
225 community. To test for those changes in community composition, we used two
226 complementary approaches: Redundancy Analysis (RDA) and log response ratio of the
227 means (LRR). The RDA analysis was of the form C ~ E where C represented the
228 Hellinger-transformed protist abundance community matrix and E the predictor matrix
229 including the effects of resource pulse from the green network (main treatment),
230 ecosystem volume in the blue network, closeness centrality (a measure of the number of
231 steps required to access every other ecosystem from a given ecosystem in the network -
232 *sensus* Freeman 1978) in the blue network, time (continuous experimental time), two-way
233 interaction between resource pulse and ecosystem volume, and two-way interaction
234 between resource pulse and time. We then ran a type 3 permutation ANOVA (999
235 permutations) to determine F-statistic and significance level for each term from the RDA
236 at $p < 0.05$. Permutations in the ANOVA were stratified by each network replicate (the 4
237 network topologies) nested within a sampling day (discrete experimental time).

238 The RDA provided a general statistical test for the effect of the predictors of interest
239 on protist community composition in the blue network, but no actual effect sizes. As a
240 second complementary step, we explored the effect of resource pulse from the green
241 network using log response ratio of the means (LRR). This approach served to confirm
242 results from the RDA and more importantly allowed us to evaluate each protist species
243 response and its effect size. The log response ratio of the mean was here defined as:

$$LRR = ln\,[\bar{x}_{R+}/\bar{x}_{R-}]$$

where ln is the natural log, $\bar{x}_{R+}$ and $\bar{x}_{R-}$ represent mean values (protist density) in the presence and absence of resource pulse, respectively. Following Hedges *et al.* (1999), the standard error of each LRR was calculated as:

$$SE[LRR] = \sqrt{\frac{s_{R+}^2}{n_{R+}\bar{x}_{R+}^2} + \frac{S_{R-}^2}{n_{R-}\bar{x}_{R-}^2}}$$

where s is the standard deviation and n is the sample size. Based on this measure of standard error we calculated confidence intervals (95%) for each LRR values. LRRs have straightforward interpretations: if the 95% confidence interval is not overlapping with 0, there is 95% probability that the population mean of effect size is indeed higher or lower than zero. Negative (or positive) LRR values means that the treatment, here resource pulse, had a negative (or positive) effect on population density.

As a complementary approach we also explored the effect of cross-ecosystem resource exchange on the community mean and standard deviation of protist traits related to body size. We used two traits measured during the video recording procedure and used for species identification: individual body length and width. Effects on community mean trait values, especially ones related to body size, can provide valuable insights on the mechanisms driving the observed changes in community composition and indicate potential, but unmeasured, impacts on ecosystem functions.

Finally, we also tested, using mixed-effect models, for effects of resource pulse from the green network on aggregate protist population and community metrics (total protist

264 densities, species richness, evenness and bioarea) in the blue network. Evenness was
265 measured as the Pielou's evenness index (Pielou 1975). Protist bioarea per mL, used as a
266 proxy of biomass, was calculated using the summed area of all individuals per video
267 frame. The mixed effect models included the interactive effects of resource pulse,
268 ecosystem volume and experimental time (continuous), and the effect of closeness
269 centrality as a co-variate. To control for temporal pseudo-replication and measure the
270 nonlinear variance associated with time (continuous time in the fixed model captures the
271 linear trend), we added each network replicate nested within experimental day (discrete
272 effect of time) as a nested random factor. The model was fitted by maximizing the
273 restricted log-likelihood ('REML', see Pinheiro *et al.* 2018). For each model we
274 decomposed the variation to evaluate the proportion of variance explained by the fixed
275 terms and random factors.

276 *Effects from blue to green network*

277 The green network was simpler in structure by design. Our main objective with the
278 green network was to test whether we can detect spatial signals of the blue dendritic
279 network on the green square lattice. Here we used a combination of a mixed-effect model
280 and log response ratios of the means to test for the effect of resource pulse from the blue
281 network, but also of the blue network structure and properties on bacteria density in the
282 green network. Our main interest was to test whether bacteria density in a connected
283 green ecosystem fluctuated depending on where in the blue network that ecosystem was
284 connected (e.g., upstream vs. downstream). The global effect of resource pulse from the
285 blue network was measured by LRR. Then as a second step, we used a mixed-effect
286 model including the effects of ecosystem volume (in the blue network), closeness

centrality (in the blue network), protist density, richness, and bioarea (in the blue network) on bacteria density in the connected green networks. To control for temporal pseudo-replication, we added experimental day (discrete effect of time) as a random factor. Again, the model was fitted by maximizing the restricted log-likelihood.

All analyses were conducted with R 3.5.1 (R Core Team 2018), using the 'bemovi' package (ver. 1.0) for video analyses (Pennekamp et al. 2015), the 'vegan' package (ver. 2.5-3) for multivariate analysis (Oksanen et al. 2018), the 'nlme' package (ver. 3.1-137) for the mixed-effect models (Pinheiro et al. 2018), the 'igraph' package (ver. 1.2.2) to extract network metrics (Csardi and Nepusz 2006), and the 'ape' (ver. 5.3) package to decompose the variation of each mixed effect models (Paradis and Schliep 2018).

Results

Testing for landscape-scale effects of cross-ecosystem resource exchange between two distinct spatial networks, we found that protist community composition from the blue networks connected to a green network by resource pulse differed from communities in isolated blue networks (Table 1, Figure 2). Resource pulse from the green network also led to changes in trait values (Figure 2) that were predictable based on species body size and species-specific response to resource pulse (Figure 3). Changes at the population level scaled up to affect aggregate community metrics related to total protist density, richness, and evenness (see Table S1 in Supporting Information). The effect of resource pulse in the blue network also varied depending on the position in the network with strongest effects found in smaller upstream ecosystems (Table 1 and Figure 4). In turn,

309 we found that bacteria density in the green network's ecosystems was significantly higher
310 when connected to a blue network (Figure 2), and this effect varied depending on how
311 central was the ecosystem in the blue network that it was connected to (Table S2, Figure
312 5).

313 More specifically, in the blue network we found an increase in community mean but
314 not in the standard deviation of trait values related to body size (mean body length and
315 width, see Figure 2) when connected to the green network. Changes in community mean
316 trait values resulted from increased population densities in the larger taxon Pca (1.2 times
317 higher, CI=[1.09,1.29], see Figures 2 and 3) with resource exchange, accompanied by
318 decreased population densities in the smaller taxa Tet (1.8 times lower, CI=[1.38, 2.25]),
319 and Chi (1.6, CI=[1.35,1.77], see Figure 2 and 3). Those variations in protist population
320 densities with resource pulse led, at the community level, to an overall decline in total
321 protist densities and richness and a marginal but significant increase in evenness in
322 connected blue networks (Table S1), but with no detectable effect on total protist bioarea
323 (a proxy for biomass, Table S1). We also found evidence that the effect of resource pulse
324 in the blue network varied with ecosystem volume ('R x V' term in Table 1) with the
325 differences between connected and isolated networks disappearing as ecosystem volume
326 increases (Figure 4).

327 In the green network, total bacteria densities were 1.26 times (CI = [1.15, 1.37]) higher
328 with than without resource pulse from the blue network (Table S1, Figure 2). This effect
329 increased with closeness centrality in the blue network (Table S2, Figure 5).

330

Discussion

The effect of cross-ecosystem resource exchange led to changes in protist population densities and varied depending on the specific spatial position of the cross-ecosystem coupling in the blue network. In the upstream ecosystems of the blue network, resource pulse from the green network favored one large taxon (*Paramecium*) but led to declines in the density of two smaller taxa (*Chilomonas*, *Tetrahymena*) compared to ecosystems in non-subsidized blue networks. In the downstream ecosystems, those effects were not detectable anymore, suggesting that the effect of cross-ecosystem resource exchange on populations diminished within the blue network in larger downstream positions. As hypothesized, we also observed a weak but significant spatial imprint ("mirroring" effect) of the blue network spatial structure on the green network. Total bacteria densities were higher in subsidized green ecosystems connected to more central ecosystems in the blue network. Consequently, localized cross-ecosystem fluxes, and their respective effects on recipient ecosystems, need to be studied from a perspective taking into account the explicit spatial configuration of the landscape.

Bacteria densities in the green network were marginally higher when connected to larger and more connected (higher centrality index) patches from the blue network. Understanding why that signal of the mirroring effect from the blue to the green network in our experiment was observed but rather weak (see Table S2 and Figure 5) can lead to insights on the processes driving cross-ecosystem dynamics at landscape extent. Two fundamental elements from our experimental design could help to explain: i) dispersal and resource exchange happened at the same frequency (two times/week), and ii) the green lattice network has a total number of links much higher than in the blue dendritic

network. In our experiment, the volume dispersed per edge was lower in the green (0.5 mL) relative to the blue (1 mL) network, however because the total number of links is higher in the green lattice network it would probably have taken a much lower dispersal volume to amplify the spatial signal and avoid homogenization. Our results suggest that dispersal needs to happen at a lower rate than resource exchange to cause a strong imprint of the connected network through spatial feedback, with the blue dendritic network imposing its own spatial constraints on the green lattice network. For metacommunities, it has been established that the balance between the speed of regional and local dynamics will drive their relative importance (e.g., mass effect vs. species sorting, Leibold *et al.* 2004; Leibold & Chase 2017). In metaecosystems, We thus propose the testable hypothesis that the balance between the speed of organism movement within a spatial network and of cross-ecosystem resource exchange might be an important metric for expectations on the regional consequences of cross-ecosystem exchanges.

The effects of cross-ecosystem resource exchange on protist population densities in the blue network scaled up to affect aggregate community metrics leading to a decline in total protist densities and species richness in ecosystems connected to the green network. Generally, the effect of resource pulse is known to be destabilizing, affecting competitive outcomes leading to decreased richness and increased dominance by a few species (Stevens et al. 2004, Cleland and Harpole 2010, Chase 2010, Hautier et al. 2014). Interestingly, in our experiment, pulse of resource from the green ecosystem varied species relative abundance but did not change the dominance ranking in the protist communities in connected compared to isolated blue networks. For instance, in the smallest ecosystems, where the effects were the strongest, *Chilomonas* remained the

dominant species despite being negatively affected by resource pulse (see Figure 4). Overall, the individual effects on each species population were strong enough to induce marginal effects on community evenness but not to shift species dominance between treatments.

The effects of cross-ecosystem resource exchange observed at the population level were still strong enough to alter community mean trait values related to body size in the community (see Figure 2). Those changes in mean trait values were associated to the density of specific taxa responding to resource exchange (see Figure 3). Cross-ecosystem fluxes selected for a larger taxa (Pca) and against the two smallest taxa (Tet, Chi). The balance between the one larger and less abundant taxon and the two smallest but more abundant taxa (see Figure 4), potentially explains why we did not observe any effect of resource pulse on protist bioarea (because of a cancelling-out differences between the two treatments). This change in community mean trait values also suggests that despite no observed change in bioarea (proxy for standing biomass) at the community level, cross-ecosystem resource exchanges can still likely affect ecosystem functions. For instance, in our experiment, subsidized ecosystems in the blue networks should have lower turnover rates due to larger individuals on average (Schramski et al. 2015) than non-subsidized ecosystems.

The relatively small effects of resource exchange observed in our experiment can be explained by different factors. Dispersal within the blue networks likely influenced the strength of the effect of localized cross-ecosystem resource exchange. It is well known that dispersal can prevent local extinctions (Hanski 1998). Especially, in our case, species that were negatively affected by cross-ecosystem resource pulse in upstream ecosystems

400 of the blue network, might have been rescued by individuals dispersing from downstream
401 ecosystems where the effect of resource pulse was not as strong. Eventually, this
402 interaction between the effect of cross-ecosystem resource exchange and within-network
403 dispersal constraints has significant and yet unexplored implications for the spatial re-
404 arrangement of communities in the landscape.

405 Evolutionary history can also influence the effect of cross-ecosystem resource
406 exchange on communities and ecosystems. A recent meta-analysis on the effect of cross-
407 ecosystem subsidies showed that experimental studies in semi-artificial systems tend to
408 have significantly lower effect sizes than observational studies (Montagano et al. 2019).
409 One explanation for those results is that experimental studies are often conducted with
410 organisms that have not necessarily evolved in an allochthonous resource pulse context
411 (Holt 2008). Moreover, experimental studies in semi-artificial systems often connect
412 systems that are very similar to one another, sharing the same evolutionary history (e.g.,
413 two protist communities), and thus also sharing similar traits (e.g., stoichiometry) making
414 it less likely to observe spatial feedback (but see Gounand *et al.* 2017a).

415 Finally, our results have implications for natural systems because they suggest that
416 upstream reaches might be more sensitive to terrestrial subsidy than larger downstream
417 reaches and this spatial dependence in the strength of the effect can spatially feedback on
418 connected ecosystems leading, at the landscape scale, to 'mirrored' dynamic and possibly
419 functioning. In our experiment, dispersal was bi-directional, while in natural systems
420 such as rivers, dispersal tends to be more unidirectional. As mentioned above, this
421 enabled rescue effect in our experiment and meant that we observed no local extinctions
422 caused by resource flows. In nature, however, directionality might lead to steeper changes

423 in community composition between upstream and downstream reaches in response to
424 cross-ecosystem exchange. Our experiment tested for the effect of landscape *per se*, all
425 else being equal. In that context, differences in the effect of resource pulse were caused
426 by intrinsic landscape characteristics: upstream patches had lower volumes and where
427 less connected compared to larger volume and more connected downstream patches.
428 Those attributes of dendritic networks were sufficient, despite the above discussed
429 ecological and evolutionary weakening factors, to observe significant impacts on
430 populations and mean community trait values, which are likely to also reflect changes in
431 ecosystem functioning. In nature, however, all else is not equal. Upstream river patches
432 are not only shallower and more isolated but they are also often more shaded, more
433 heterotrophic, and more prone to large seasonal pulse caused by mobile animals moving
434 against the flow (e.g., anadromous salmon) and thus potentially more dependent on
435 allochthonous subsidies than larger downstream patches (England and Rosemond 2004).
436 Therefore, our results are conservative because in natural systems upstream patches are
437 smaller and receive more subsidies, all else being equal.

438 Taken together, our results show how metaecosystem dynamics can impact the
439 balance of community composition and trigger cross-ecosystem feedbacks that can
440 spread across a whole landscape. Our study thus constitutes another illustration of the
441 need to integrate spatial structure into land management, and confirm the need to
442 incorporate more of the complexity of natural landscape into meta-ecosystem theory
443 (Leroux et al. 2017, Gounand et al. 2017b).

444

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References

Altermatt, F. and Fronhofer, E. A. 2018. Dispersal in dendritic networks: Ecological consequences on the spatial distribution of population densities. - Freshw Biol 63: 22–32.

Altermatt, F. et al. 2015. Big answers from small worlds: a user's guide for protist microcosms as a model system in ecology and evolution. - Methods Ecol Evol 6: 218–231.

Bultman, H. et al. 2014. Terrestrial deposition of aquatic insects increases plant quality for insect herbivores and herbivore density. - Ecol Entomol 39: 419–426.

Carrara, F. et al. 2012. Dendritic connectivity controls biodiversity patterns in experimental metacommunities. - PNAS 109: 5761–5766.

Carrara, F. et al. 2014. Complex Interaction of Dendritic Connectivity and Hierarchical Patch Size on Biodiversity in River-Like Landscapes. - The American Naturalist 183: 13–25.

Chase, J. M. 2010. Stochastic community assembly causes higher biodiversity in more productive environments. - Science 328: 1388–91.

Cleland, E. E. and Harpole, W. S. 2010. Nitrogen enrichment and plant communities. - Annals of the New York Academy of Sciences 1195: 46–61.

Csardi, G. and Nepusz, T. 2006. The igraph software package for complex network research. - InterJournal Complex Systems: 1695.

England, L. E. and Rosemond, A. D. 2004. Small reductions in forest cover weaken terrestrial-aquatic linkages in headwater streams. - Freshwater Biology 49: 721–734.

Finn, D. S. et al. 2011. Small but mighty: headwaters are vital to stream network biodiversity at two levels of organization. - Journal of the North American Benthological Society 30: 963–980.

Fisher, S. G. and Likens, G. E. 1973. Energy flow in Bear Brook, New Hampshire: An integrative approach to stream ecosystem metabolism. - Ecological Monographs 43: 421–439.

Freeman, L. C. 1978. Centrality in social networks conceptual clarification. - Social Networks 1: 215–239.

Gounand, I. et al. 2017a. Subsidies mediate interactions between communities across space. - Oikos 126: 972–979.

Gounand, I. et al. 2017b. Meta-Ecosystems 2.0: Rooting the Theory into the Field. - Trends in Ecology & Evolution 33: 36–46.

Hanski, I. 1998. Metapopulation dynamics. - Nature 396: 41–49.

Hanski, I. and Gaggiotti, O. E. 2004. Metapopulation biology: past, present, and future. - In: Hanski, I. and Gaggiotti, O. E. (eds), Ecology, genetics, and evolution of metapopulations. Academic Press, pp. 3–22.

Harvey, E. and MacDougall, A. S. 2014. Trophic island biogeography drives spatial divergence of community establishment. - Ecology 95: 2870–2878.

Harvey, E. et al. 2016. Spatially cascading effect of perturbations in experimental meta-ecosystems. - Proc. R. Soc. B 283: 20161496.

Harvey, E. et al. 2017. Upstream trophic structure modulates downstream community dynamics via resource subsidies. - Ecol Evol 7: 5724–5731.

Harvey, E. et al. 2018. Disturbance reverses classic biodiversity predictions in river-like landscapes. - Proceedings of the Royal Society B: Biological Sciences 285: 20182441.

Hautier, Y. et al. 2014. Eutrophication weakens stabilizing effects of diversity in natural grasslands. - Nature 508: 521–525.

Hedges, L. V. et al. 1999. The Meta-Analysis of Response Ratios in Experimental Ecology. - Ecology 80: 1150–1156.

Holt, R. D. 2008. Theoretical Perspectives on Resource Pulses. - Ecology 89: 671–681.

2005. Metacommunities: spatial dynamics and ecological communities (M Holyoak, MA Leibold, RD Holt, and Ecological Society of America, Eds.). - University of Chicago Press.

Knight, T. M. et al. 2005. Trophic cascades across ecosystems. - Nature 437: 880–883.

Koel, T. M. et al. 2019. Predatory fish invasion induces within and across ecosystem effects in Yellowstone National Park. - Science Advances 5: eaav1139.

Lafage, D. et al. 2019. Local and landscape drivers of aquatic-to-terrestrial subsidies in riparian ecosystems: a worldwide meta-analysis. - Ecosphere 10: e02697.

Leibold, M. A. and Chase, J. M. 2017. Metacommunity ecology. - Princeton University Press.

Leibold, M. A. et al. 2004. The metacommunity concept: a framework for multi-scale community ecology. - Ecology Letters 7: 601–613.

518 Leroux, S. J. and Loreau, M. 2012. Dynamics of Reciprocal Pulsed Subsidies in Local
519 and Meta. - Ecosystems 15: 48–59.

520 Leroux, S. J. et al. 2017. Stoichiometric distribution models: ecological stoichiometry at
521 the landscape extent. - Ecol Lett 20: 1495–1506.

522 Montagano, L. et al. 2019. The strength of ecological subsidies across ecosystems: a
523 latitudinal gradient of direct and indirect impacts on food webs. - Ecology Letters
524 22: 265–274.

525 Mucha, P. J. et al. 2010. Community Structure in Time-Dependent, Multiscale, and
526 Multiplex Networks. - Science 328: 876–878.

527 Oksanen, J. et al. 2018. vegan: Community Ecology Package.

528 Paradis, E. and Schliep, K. 2018. ape 5.0: an environment for modern phylogenetics and
529 evolutionary analyses in R. - Bioinformatics 35: 526–528.

530 Pennekamp, F. and Schtickzelle, N. 2013. Implementing image analysis in laboratory-
531 based experimental systems for ecology and evolution: a hands-on guide. -
532 Methods Ecol Evol 4: 483–492.

533 Pennekamp, F. et al. 2015. BEMOVI, software for extracting behavior and morphology
534 from videos, illustrated with analyses of microbes. - Ecol Evol 5: 2584–2595.

535 Pielou, E. C. 1975. Ecological diversity. - Wiley.

536 Pinheiro, J. et al. 2018. nlme: Linear and Nonlinear Mixed Effects Models.

537 Polis, G. A. and Hurd, S. D. 1995. Extraordinarily high spider densities on islands: flow
538 of energy from the marine to terrestrial food webs and the absence of predation. -
539 PNAS 92: 4382–4386.

540 Polis, G. A. et al. 1997. Toward an integration of landscape and food web ecology:The
541 Dynamics of Spatially Subsidized Food Webs. - Annual Review of Ecology and
542 Systematics 28: 289–316.

543 Richardson, J. S. and Sato, T. 2015. Resource subsidy flows across freshwater–terrestrial
544 boundaries and influence on processes linking adjacent ecosystems. - Ecohydrol.
545 8: 406–415.

546 Rigon, R. et al. 1993. Optimal channel networks: A framework for the study of river
547 basin morphology. - Water Resour. Res. 29: 1635–1646.

548 Rinaldo, A. et al. 2006. Trees, networks, and hydrology. - Water Resour. Res. 42:
549 W06D07.

550 Rodriguez-Iturbe, I. and Rinaldo, A. 1997. Fractal River Basins: Chance and Self-
551 Organization. - Cambridge University Press.

552 Schindler, D. E. and Smits, A. P. 2017. Subsidies of Aquatic Resources in Terrestrial
553 Ecosystems. - Ecosystems 20: 78–93.

554 Schramski, J. R. et al. 2015. Metabolic theory predicts whole-ecosystem properties. -
555 PNAS 112: 2617–2622.

556 Soininen, J. et al. 2015. Toward more integrated ecosystem research in aquatic and
557 terrestrial environments. - BioScience: biu216.

558 Stevens, C. J. et al. 2004. Impact of nitrogen deposition on the species richness of
559 grasslands. - Science 303: 1876–1879.

560 Tonkin, J. D. et al. 2018a. Flow regime alteration degrades ecological networks in
561 riparian ecosystems. - Nature Ecology & Evolution 2: 86–93.

562 Tonkin, J. D. et al. 2018b. Metacommunities in river networks: The importance of
563 network structure and connectivity on patterns and processes. - Freshw Biol 63:
564 1–5.

565 Tscharntke, T. et al. 2012. Landscape moderation of biodiversity patterns and processes -
566 eight hypotheses. - Biological Reviews 87: 661–685.

567

568

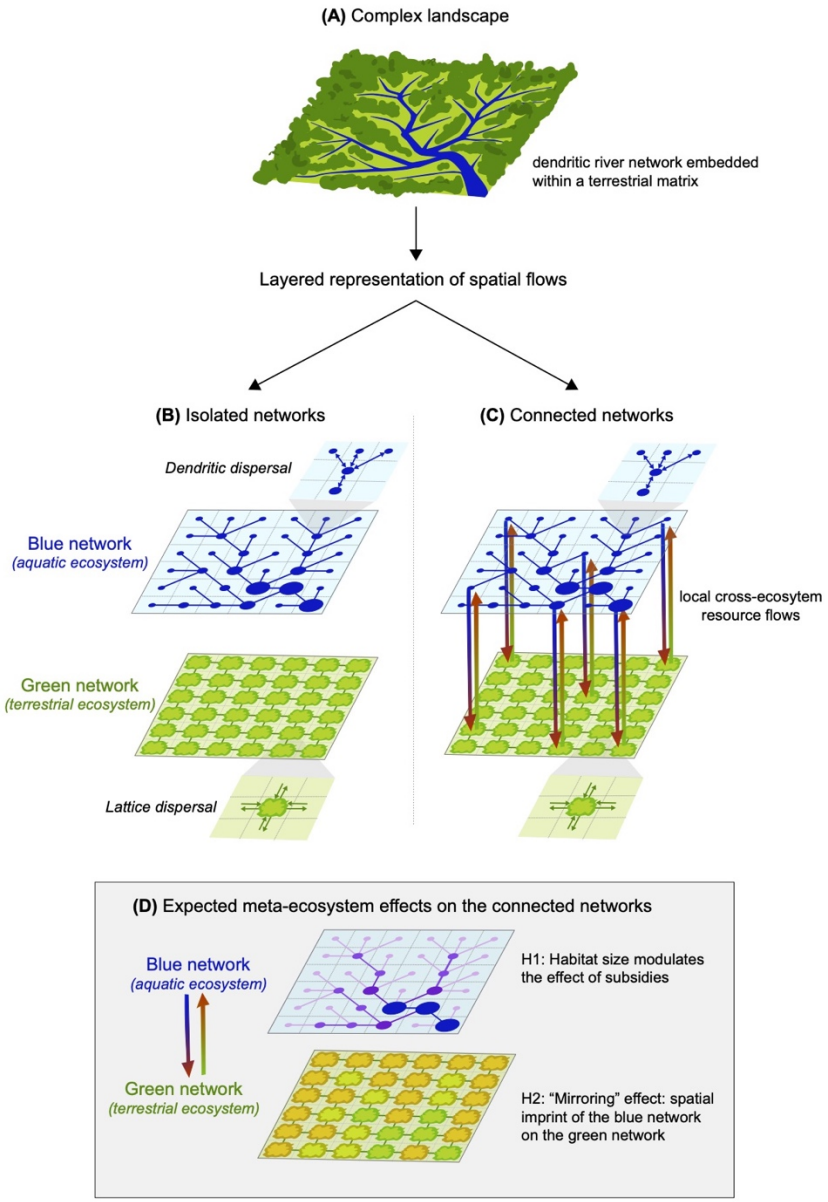
569

570 **Table 1.** Permutation ANOVA (999 permutations) on the Redundancy Analysis model
571 used to test effects on protist community composition (abundance and occurrence) in the
572 blue network.
573

	Df	F
Resource pulse (R)	1	49.97***
Ecosystem volume (V)	1	59.20***
Centrality	1	12.21***
Time (T)	1	237.65***
R x V	5	2.38***
R x T	5	15.65***
Residuals	1137	

574 '***' 0.001; '**' 0.01; '*' 0.05
575
576
577

578 **Figure 1**

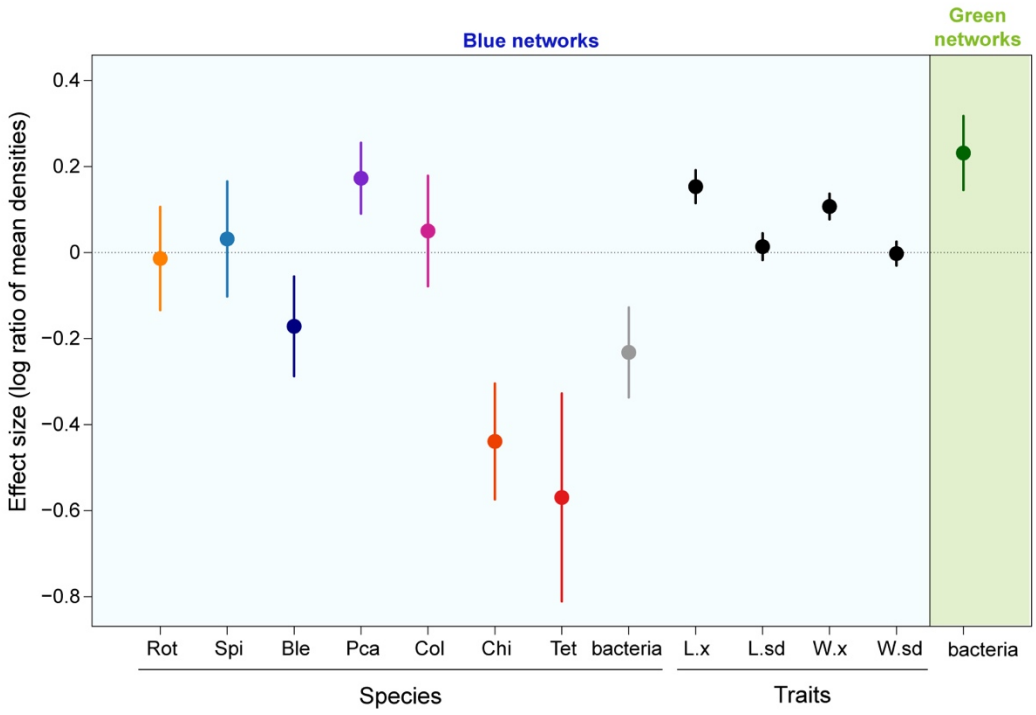


579

580 **Figure 1.** Conceptual representation of the experimental design. (**A**) Natural landscapes
581 are composed of dendritic river networks embedded within terrestrial matrices. This
582 complexity can be decomposed using a layered representation with two distinct spatial
583 networks: an aquatic dendritic network and a terrestrial lattice network. (**B**) For each
584 spatial network dispersal occurs along the edges, (**C**) while cross-ecosystem resource

585 exchange is local and links each node in the respective position in the two networks (only
586 few arrows drawn, for clarity). (**D**) Hypotheses on the interactive effects of cross-
587 ecosystem resource exchange and spatial structure on green and blue networks.

588 **Figure 2.**



589

590 **Figure 2.** Effect size of the cross-ecosystem resource pulse respectively on protist taxa
591 and bacteria density, and on community mean and standard variation of trait values in the
592 blue network, and finally on bacteria density in the green network. Each point represents
593 the log ratio of the mean effect of the treatment (as described in the Methods section).
594 Error bars represent the 95% confidence interval calculated based on the LRR standard
595 error formula described in the Methods section. An LRR higher than zero represents a
596 positive effect of resource pulse on density and a negative LRR, the opposite. For body
597 length (L) and width (W), x represents the mean and sd the standard variation of the mean
598 trait value. The different protist taxa are color coded and named by their abbreviations
599 described in the Methods section.

600

Figure 3.

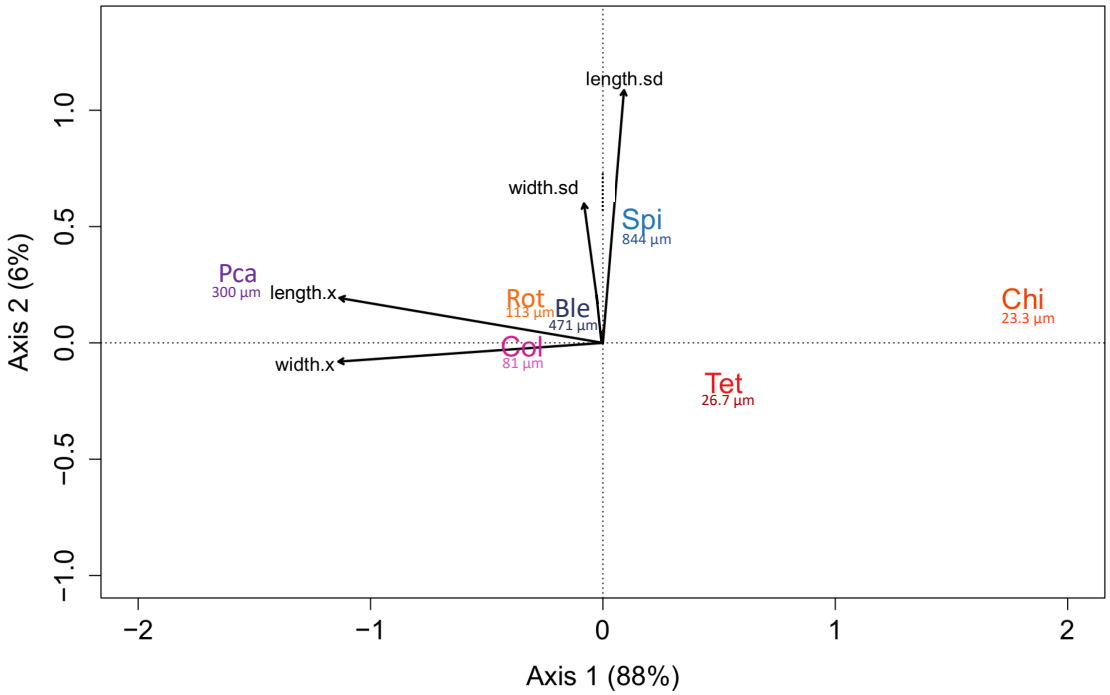


Figure 3. Redundancy analysis showing the association between community mean trait values and protist taxa density in the blue network. Values for each taxon represent average body size taken from the literature. The figure shows that larger taxon Pca is more abundant in communities with higher averaged body size, while smaller taxa Tet and Chi are more abundant in communities with lower averaged body size. The different protist taxa are color coded and named by their abbreviations described in the Methods section.

Figure 4.

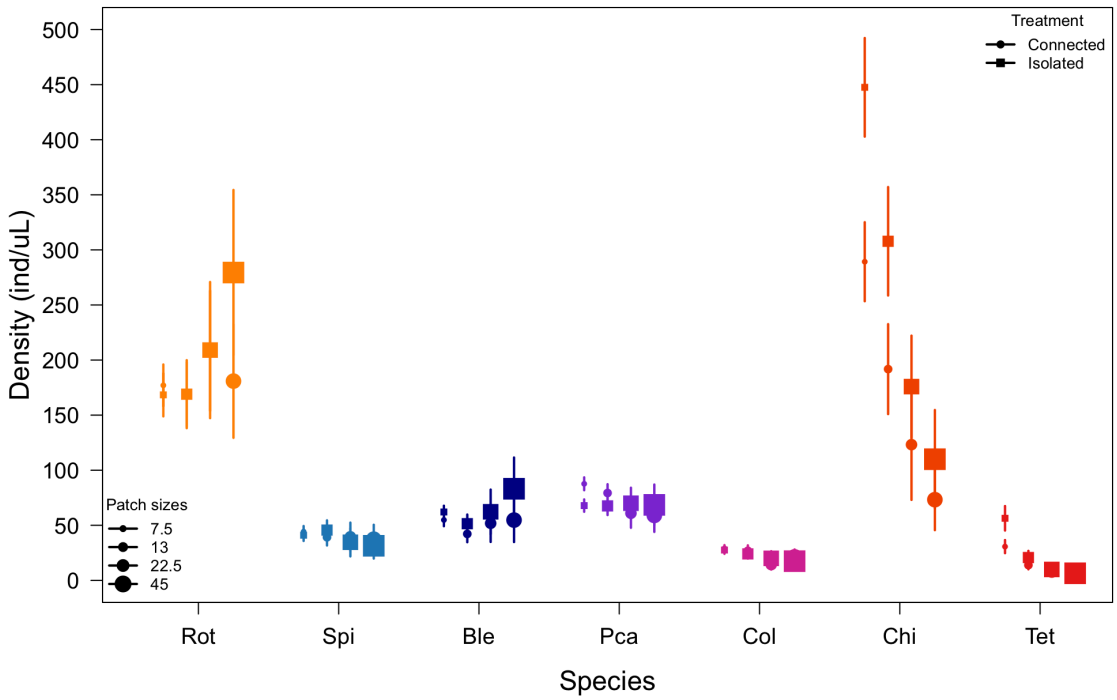
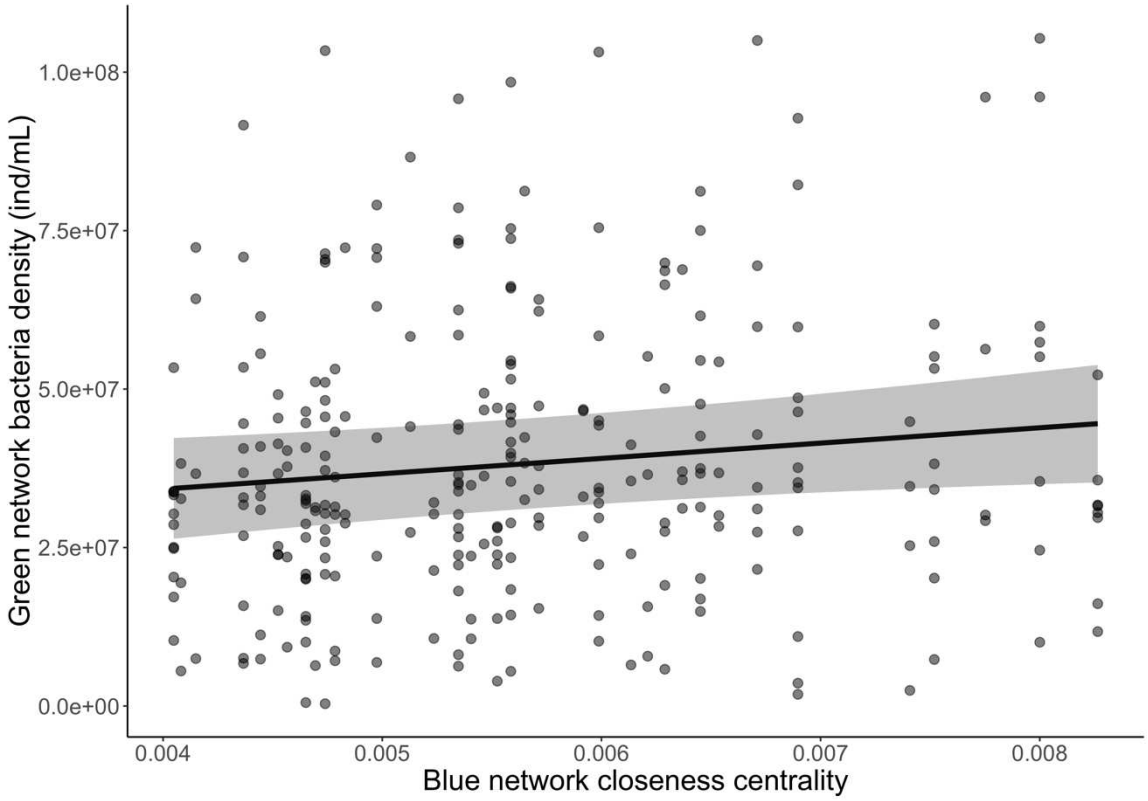


Figure 4. Effect of the cross-ecosystem resource pulse treatment on protist taxa density for the different ecosystem volumes (patch sizes) in the blue network. Each point represents the mean density and error bars represent the 95% confidence intervals. The figure shows that the effects of resource exchange on Ble, Pca, Chi and Tet shown in Figure 1 tend to disappear in larger patch sizes. The different protist taxa are color coded and named by their abbreviations described in the Methods section.

623 **Figure 5.**

624



625

626 **Figure 5.** Effect of closeness centrality in the blue network on bacteria density in the
627 connected green networks. Points represent the raw data. The black line is the prediction
628 from the mixed effect model (Table S2), and shaded area represents the 95% confidence
629 interval. Marginal R^2 is 0.03 (centrality effect only), conditional R^2 is 0.09 (centrality +
630 random effects). Spearman correlation between centrality and bacteria density is 0.13 (p-
631 value = 0.02).